REMARKS/ARGUMENTS

Claims 1, 3, 5, 6 and 14-20 are pending in the application. Claim 1 was amended to "clarify" the phrase "a first test substance and... a second test substance" in step (D)." No new matter has been added by virtue of this amendment and entry is respectfully requested. The cancellation of subject matter is not to be construed as a surrender of any subject matter. Applicants hereby reserve the right to pursue any canceled or amended subject matter in one or more continuation or divisional applications.

Claim Rejections Under 35 U.S.C. §112

Claims 1, 3, 5, 6, and 14-20 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Applicants respectfully traverse. However, in order to compact and expedite prosecution, Applicants have amended claim 1 which clarifies "a first test substance and... a second test substance" in step (D). The Examiner asserts that it is "unclear if this the first test substance and the second test substance of step (A) or if this is another two test substances." Although Applicants disagree with this assessment, Applicants have amended claim 1 to recite that the substances obtained from the library of test compounds are tested in step (D). The amendment is solely for the purpose of responding to this office action and to expedite prosecution. The amendment is not to be considered as surrender of any subject matter. Applicants hereby reserve the right to pursue any canceled or amended subject matter in one or more continuation or divisional applications.

Claims 3, 5, 6 and 14-20 are rejected for depending from a rejected base claim and not overcoming the clarity issues of such base claim. The amendment of claim 1, from which claims 3, 5, 6 and 14-20 depend from renders this rejection moot.

In view thereof, Applicants respectfully request reconsideration and withdrawal of the instant rejection.

Claims 1, 3, 5, 6, and 14-20 remain rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement, for reasons of record. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Applicants respectfully traverse.

The Examiner asserts:

> Claim 1, from which all the other pending claims are dependent, and therefore encompass, encompasses a method for identifying any drug candidate for promoting tissue-specific differentiation of an embryonic stem cell into any type of cell, with specific method steps comprising a pre-culturing step for two days (step B) and a culturing step of at least about *5* days on a collagen coated culture plate (step c). Claims 3, 5, 6, and 14-20 are also rejected, as they do not modify the method in such a manner to allow possession, for the reasoning below.

Applicants describe the screening assay using murine embryonic stem cells (also shown in Figure 1), for example, on page 12, lines 24-30 through to page 13, lines 1-3:

> Example 1- Screening Assay Using Murine Embryonic Stem Cells
> An overview of one method of the invention is presented in FIG. 1. 300 mouse ES cells in differentiating medium are added to wells of a 96 well microtiter plate. The plate is cultured for seven days under conditions that promote differentiation of ES cells. Ninety-six different substances from a library (e.g., a chemical compound library) are then separately added, one substance to a well, to the wells of the plate. The plate is returned to culture for an additional 7-14 days. After this period, total RNA is extracted from each well of the plate. The extracted RNA is then evaluated for increased expression of tissue specific mRNAs (e.g. alpha cardiac myosin-heavy chain mRNA for cardiac myocyte-specific differentiation, albumin mRNA for hepatocyte-specific differentiation,

> etc.). Methods for evaluating mRNA expression include RT-PCR, dot-blot, and cDNA/gene chip technology.

Applicants describe the use of a library, i.e. "for identifying any drug candidate." The addition of any drug compound is tested in the assay (e.g." [n]inety-six different substances from a library (e.g., a chemical compound library) are then separately added, one substance to a well, to the wells of the plate). Applicants further teach "extracted RNA is then evaluated for increased expression of tissue specific mRNAs."

Applicants also teach the pre-culturing step for two days (step B) and a culturing step of at least about 5 days on a collagen coated culture plate (step C). See, for example, page 13, lines 9-22:

> Cell Culture-The ES cell lines R1 (129Sv strain), W9.5 (129Sv), and SEK1 null (established from W9.5) (Ganiatsas et al., (1998) Proc. Natl. Acad. Sci. USA 95, 6881-6) were maintained undifferentiated in gelatin-coated dishes in DMEM (GIBCO BRL, Grand Island, NY) containing 15% fetal bovine serum (Atlanta biologicals, Norcross, GA), 2 mM L-glutamine, 100 units/ml penicilin, 100 μg/ml streptomycin, 25 mM Hepes (GIBCO BRL), 300 μM monothioglycerol (Sigma, St. Louis, MO), and 250 unit/ml recombinant mouse LIF (ESGRO, CHEMICON, Temecula, CA). To induce differentiation, ES cells were suspended in IMDM containing 2 mM L-glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin (GIBCO BRL), 20% fetal bovine serum (Atlanta biologicals) and 300 μM monothioglycerol (Sigma). **Cells were cultured for 2 days** by the hanging-drop method (1×10^3 ES cells per 30 μl in each drop) (Metzger et al., (1994) J. Cell. Biol. 126, 701-11). EBs in hanging drops were transferred to **suspension culture in 100-mm petri dishes and cultured for an additional 3 days.** The resulting EBs were **plated onto six-well tissue culture dishes coated with or without Vitrogen (collagen type I)** (COHESION, Palo Alto, CA). (Emphasis added).

After these cells are plated, they are used in the experiments, i.e. screening assay as described on page 13, lines 22-26. Figure 2 depicts the steps described in the above passage. Applicants submit that claim 1 meets the requirements of 35 U.S.C. § 112, paragraph 1. In

addition, claims 3, 5, 6 and 14-20 depend on claim 1 and as such encompass all the limitations of claim 1.

In view thereof, Applicants respectfully request reconsideration and withdrawal of the instant rejection.

Claim Rejections 35 U.S.C. § 112 – enablement

Claims 1, 3, 5-6, and 14-20 remain rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for the identification of substances which cause EBs to differentiate into the hepatic lineage, does not reasonably provide enablement for a method to screen for substances which cause differentiation into any lineage from EBs, or any tissue specific lineage from ES cells, for reasons of record, as elaborated upon below. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

Applicants respectfully traverse.

The Examiner asserts that the invention is directed to hepatocyte differentiation only "because the collagen at step (C) appears to induce differentiation into hepatocytes, the Artisan could not reasonably predict any other cell type could be formed, as the collagen may overpower any other differentiation pathway." Applicants teach that the cells were plated in culture plates coated with collagen and in plates **lacking** collagen. These two sets of cultures were then used to test the library of test compounds and identify whether the stem cells differentiate. See, for example, page 13, lines 22-26:

> The resulting EBs were plated onto six-well tissue culture dishes coated **with or without** Vitrogen (collagen type I) (COHESION, Palo Alto, CA). In some experiments, the growth factors were added into culture medium (100 ng/ml acidic fibroblast growth factors (aFGF), 20 ng/ml hepatocyte growth factor (HGF), 10 ng/ml oncostatin M, with 10^{-7} M dexamethasone

> (Sigma), and ITS (5 mg/ml insulin, 5 mg/ml transferrin, 5 μg/ml selenious acid, Collaborative Biomedical Products, Benford, MA)). (Emphasis added).

Thus, Applicants teach that the experiments include cells which were tested in tissue culture plates lacking collagen. These cells could, therefore, differentiate into lineages of cell types other than hepatocytes, because they would not, as asserted by the Examiner be "far down the tree of differentiation." Further, Applicants have amended claim 1 to indicate and clarify that some cells were cultured in collagen coated plates and others in plates without collagen. As such, any compound can be tested without undue experimentation and determine whether that compound induces differentiation of the EBs, coupled with the level of skill in the art and the prior art at the time the application was filed, enables one skilled in the art to make and use the claimed invention. For these reasons, the enablement requirement of 35 U.S.C. § 112 for each of the pending claims has been satisfied. Accordingly, withdrawal of these rejections and allowance of all pending claims is respectfully requested.

CONCLUSION

Applicants respectfully request entry of the foregoing amendments and remarks and reconsideration and withdrawal of all rejections. It is respectfully submitted that this application is in condition for allowance. If there are any remaining issues or the Examiner believes that a telephone conversation with the Applicants' attorney would be helpful in expediting prosecution of this application, the Examiner is invited to call the undersigned at telephone number shown below.

Although, Applicants believe that no extensions of time are required with submission of this paper, Applicants request that this submission also be considered as a petition for any extension of time if necessary. The Commissioner for Patents and Trademarks is hereby authorized to charge the amount due for any retroactive extensions of time and any deficiency in any fees due with the filing of this paper or credit any overpayment in any fees paid on the filing or during prosecution of this application to Deposit Account No. 50-0951.

Respectfully submitted,
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